

**Final Minutes of the National Toxicology Program (NTP) Advisory Committee on
Alternative Toxicological Methods (ACATM) Meeting**

March 4, 1999

**National Institute of Environmental Health Sciences (NIEHS)
Building 101
Research Triangle Park, NC**

The following ACATM members were in attendance:

- Katherine A. Stitzel, D.V.M. (Chair), Procter & Gamble Company, Cincinnati, Ohio
- Paul T. Bailey, Ph.D., Mobil Business Resources Corporation, Paulsboro, New Jersey
- Michael S. Denison, Ph.D., University of California—Davis, Davis, California
- Elaine Faustman, Ph.D., University of Washington, Seattle, Washington
- Sidney Green, Ph.D., Howard University College of Medicine, Washington, D.C.
- A. Wallace Hayes, Ph.D., Gillette Company, Boston, Massachusetts
- Susan Hurt, Ph.D., Rohm and Haas Company, Spring House, Pennsylvania
- Roger McClellan, D.V.M., Chemical Industry Institute of Toxicology, Research Triangle Park, NC
- Charles Montgomery, D.V.M., Baylor College of Medicine, Houston, Texas
- Peter Theran, D.V.M., Massachusetts Society for the Prevention of Cruelty to Animals, Boston, Massachusetts

The following ACATM members were absent:

- Alan M. Goldberg, Ph.D., Johns Hopkins University, Baltimore, Maryland
- Andrew N. Rowan, Ph.D., Humane Society of the United States, Gaithersburg, Maryland

Other Meeting Attendees:

Ms. Loretta Brammell, NIEHS/ NTP Center	Dr. William Stokes, NIEHS/NTP Center
Dr. John Bucher, NIEHS	Ms. Heather Vahdat, ILS, Inc./NTP Center
Dr. Thomas Goldsworthy, ILS, Inc./ NTP Center	Dr. Neil Wilcox, Food and Drug Administration
Ms. Karen Haneke, ILS, Inc./NTP Center	Dr. Barry Margolin, ILS, Inc./NTP Center
Dr. Larry Hart, NIEHS	Mr. George Clark, XDS
Dr. George Lucier, NIEHS	Ms. Mary Beth Sweetland, PETA
Ms. Debbie McCarley, NIEHS/NTP Center	Mr. Eric Wilson, PETA
Ms. Denise Sailstad, U.S. Environmental Protection Agency	Dr. J.C. Bhandari, Novel
	Ms. Ella Budgraf, Unilever
	Dr. William Mundy, UNHEERL, U.S. EPA

Call to Order and Introductions

Dr. Stitzel called the meeting to order at 8:45 a.m., and asked those in attendance to state their name and affiliation for the record. Dr. Hart asked that individuals use the microphone when speaking, and state his or her name and affiliation for the record before each comment. He further asked that all attendees register at the table located directly outside the meeting room. Dr. Hart then briefly reviewed conflict of interest issues that are pertinent to the NTP, and reminded ACATM members that they are serving as individual scientists at the meeting, not as representatives of a company or organization.

Welcome and NTP Update

Dr. Lucier welcomed everyone to the second ACATM meeting, and briefly reviewed the progress of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the NTP Interagency Center for the Validation of Alternative Toxicological Methods (NICEATM; NTP Center) since the last meeting. Two test method peer reviews have been successfully conducted, and much has been learned about the process. Dr. Lucier stated that the purpose of the ACATM meeting was for the members to provide input on the process and suggestions for potential improvements, and to discuss priorities and issues for ICCVAM and the NTP Center to consider in the future. He mentioned two topics that will involve ICCVAM, both of which are currently under consideration at the EPA: the endocrine disruptor screening and testing initiative and the high production volume (HPV) chemical testing program.

Dr. Lucier then provided an update on the activities of the NTP. He stated that the focus of all activities is mechanism-based toxicology and the development of potential applications. RO3 grants (small research grants), which are available to universities, are being used to help improve these mechanism-based evaluations. Other activities of the NTP include the environmental genome project, investigations on oxidative stress, receptor-mediated toxicology studies, the annual Report on Carcinogens, a workshop on herbal medicines, evaluations of drinking water disinfectant byproducts, and establishment of the Center for the Evaluation of Risks to Human Reproduction (CERHR).

Dr. Green asked if the CERHR focused on reproductive development, and Dr. Lucier answered in affirmative. Dr. Stitzel asked if this Center will be conducting data reviews only or if they would also identify research needs. Dr. Lucier replied that the CERHR will produce narrative statements on potential human reproductive toxicants based on what is known about the substance and its effects, and that these reports will serve to identify research needs and knowledge gaps.

Update on ICCVAM and the NTP Center

Dr. Stokes provided an update on the activities of ICCVAM and the NTP Center. Three test methods have been or are being considered. These include the murine Local Lymph Node assay (LLNA), Corrositex[®], and the Frog Embryo Teratogenesis Assay *Xenopus* (FETAX). The LLNA was submitted to ICCVAM for consideration in February, 1998, by Drs. Frank Gerberick (Procter & Gamble), David Basketter (Unilever), and Ian Kimber (Zeneca). The public peer review meeting took place on September 17, 1998. The peer review evaluation report, "The Murine Local Lymph Node Assay: A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals/ Compounds,"¹ was forwarded with ICCVAM recommendations to Federal agencies for their consideration on February 18, 1999.

Using the LLNA as an example, Dr. Stokes provided an overview of the peer review process. Following receipt of the submission, ICCVAM assembled a working group (the Immunotoxicity Working Group [IWG]) of Federal agency representatives with relevant expertise. The role of the working group is to supplement the test method submission guidelines as appropriate for the method under consideration, ensure that adequate test method information is available, identify and recommend expert peer review panel (Panel) members, develop peer review questions appropriate to the method, comment on the Panel report, and prepare recommendations for agencies on potential applications of the method. The role of the LLNA Panel was to evaluate the extent to which the submission addressed the ICCVAM validation and regulatory acceptance criteria, and to develop a consensus on the usefulness of the method as a stand-alone alternative to currently accepted assays for assessing allergic contact dermatitis. Following receipt of the Panel report, regulatory agencies will determine if the method is applicable and acceptable for their regulatory mandates. Similarly, Federal non-regulatory agencies will determine if the LLNA has potential applications, and if so, they will need to inform appropriate scientists and other individuals.

Dr. Stokes made a few brief comments about Corrositex[®], the second assay considered by ICCVAM. More detailed information was presented later in the meeting by NTP Center staff and Panel members.

Dr. Stokes mentioned the status of FETAX, the third assay under consideration by ICCVAM. The Environmental Protection Agency (EPA) requested a review of the validation status of this developmental toxicity screening method. A Background Review Document that summarizes the validation status of FETAX, including all currently available test method data and performance information, is being prepared by the NTP

¹ "The Murine Local Lymph Node Assay: A Test Method for Assessing Allergic Contact Dermatitis Potential of Chemicals/Compounds," NIH Publication 99-4494, February 1999. National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA. Available on the Internet, <http://ICCVAM.NIEHS.NIH.GOV/llnarep.htm>

Center in accordance with the ICCVAM submission guidelines². A review meeting is anticipated for Fall, 1999 following completion of the background review document.

Dr. Stokes also reviewed the purpose of the ICCVAM test method submission guidelines. The guidelines were developed to provide a format for test method submissions and background review documents, and to describe the types of data and information needed by ICCVAM to assess the current validation status of a test method. The guidelines are applicable to any stage of test method development and validation, and additional guidance may be added as appropriate for specific methods. He highlighted the NTP Center support activities, which include: providing operational support to ICCVAM, pre-screening of test method submissions, preparing test method background review documents, organizing test method peer reviews/workshops, preparing documents for publication, and providing information services.

Dr. Stokes briefly mentioned several test methods that may be considered in the future by ICCVAM. These include endocrine disruptor screening and testing methods, methods used to characterize the toxicity of High Production Volume (HPV) chemicals, transgenic models for carcinogenicity, the HCE-7 ocular irritancy assay, and the mouse ear swelling test for allergic contact dermatitis.

Dr. Stokes updated the committee on Organization for Economic Cooperation and Development (OECD)-related activities. OECD is preparing a guidance document on validation, as well as a guidance document on humane endpoints in toxicity testing. The initial draft guidance document on humane endpoints was completed in October, 1998 and reviewed at an expert meeting in November, 1998. Completion of a revised draft that will be circulated for comments by participating countries is anticipated in Spring, 1999. OECD is also preparing a guidance document on acute oral toxicity, which addresses appropriate use of the four current OECD test guidelines for acute toxicity testing. An OECD expert meeting on this issue, hosted by EPA, will take place on March 22 to 24, 1999.

Dr. Theran asked about the mission of the EPA Endocrine Disruptor Screening and Testing Standardization and Validation Task Force. Dr. Stokes replied that the Task Force is responsible for standardizing and validating screening and testing methods based on the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) report, which developed recommendations for screening and testing methods. In the report, it was recommended that if chemicals are evaluated using the screening tests and negative results are obtained, no further testing would normally be required. If positive results are obtained in the screening tests, then one or more definitive tests would be

² "Validation and Regulatory Acceptance of Toxicological Test Methods: A Report of the ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods," NIH Publication 97-3981, March 1997. National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA. Available on the Internet, <http://ntp-server.niehs.nih.gov/htdocs/ICCVAM/iccvam.html>.

necessary. As mandated by Congress, the endocrine disruptor screening and testing program must be in place by August, 1999.

Dr. Lucier added that EDSTAC identified 16 methods, some of which are well known (e.g., the reporter gene assays), and others that are not. The Task Force is charged with standardizing and validating each of these methods. Dr. Theran asked if the EPA was creating its own committee charged with the same tasks as those of ICCVAM. He mentioned that many organizations are hoping that ICCVAM will review the validation status of endocrine disruptor screening and testing methods. Dr. Lucier stated that EPA officials are working with Dr. Stokes to ensure that the proposed methods are reviewed using the ICCVAM peer review process.

Dr. Hurt asked how the validation efforts for endocrine disruptor screening and testing will be shared between EPA, ICCVAM, and OECD. Dr. Stokes stated that the three organizations will need to work together to eliminate redundant efforts. OECD validation studies for two mammalian methods (i.e., the Hershberger Assay and the Uterotrophic Assay) will likely meet the needs of EPA, and therefore EPA is not planning to conduct independent studies. Dr. Hurt asked if the validation efforts for those two assays will be conducted by OECD rather than by ICCVAM; Dr. Stokes confirmed that this was the situation.

Dr. Faustman asked if the extent of guidance provided to submitters on test method performance assessment and the selection of reference chemicals were sufficient. Dr. Stokes replied that the submission guidelines are not considered to be stand-alone, and that the introductory paragraph refers test developers to the 1997 publication entitled, "Validation and Regulatory Acceptance of Toxicological Test Methods: A Report of the ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods," NIEHS Publication No. 97-3981. This report contains specific information on how to selection of both test chemicals and control chemicals, and specifies that the test developers need to test a range of possible chemical and/or product classes. Dr. Faustman speculated on whether the ICCVAM submission guidelines were overly rigorous, causing some investigators to not submit their assay for consideration to ICCVAM. Dr. Stokes replied that this does not seem to be an issue at this point, and that ICCVAM is working to foster communication with potential submitters early in the validation process. By doing so, ICCVAM can communicate with the participating agencies to determine what information they would expect to see generated in validation testing. This information would then be provided to the test developer.

Ms. Sailstad commented on the useful role that ICCVAM could serve by convening workshops and expert meetings to optimize test methods before large-scale validation studies are conducted. Dr. Stokes added that some peer reviews will be conducted for assays that are in intermediate stages of validation, and the purpose of such workshops/expert meetings will be to provide guidance for future validation efforts.

Dr. Stitzel mentioned that a review of the historical data to which a new assay is compared would be a productive topic for a future workshop/expert meeting. The workshop/expert meeting could focus on the ability of data from previously accepted test methods to accurately predict human response. Dr. Stokes stated that human data is important, but that in some cases such data is not available. An example is corrosivity, where human testing cannot ethically take place and only accidental exposure case reports are available. Dr. Stokes added that the ICCVAM submission guidance states that any available human data should be provided, and that the performance of the new and the existing test methods should be compared against this human data.

The LLNA Peer Review Report and ICCVAM Recommendations

Ms. Sailstad gave an update on the Immunotoxicity Working Group (IWG) perspective of the LLNA peer review process. She briefly reviewed the conclusions of the Panel meeting, the IWG, and ICCVAM. Ms. Sailstad reiterated the challenges facing the IWG as well as future working groups, which are to maintain the focus of the review, develop recommendations for ICCVAM, and to facilitate communication among the Sponsor, the NTP Center, ICCVAM, and the Panel. At the public LLNA Panel meeting held on September 17, 1998, the Panel concluded that the LLNA had been evaluated sufficiently and its performance was satisfactory to support its adoption as a stand-alone alternative to guinea pig tests, with certain considerations. These considerations included the use of the proposed mouse strain and gender until others strains and male mice have been appropriately evaluated, collection of data from individual animals rather than from samples pooled across animals within a dose group, and the recording of body weights. The Panel also concluded that the LLNA offered advantages with respect to animal welfare considerations. Other Panel recommendations included conducting a retrospective data audit, modifying the protocol to include the considerations listed above, and allowing the use of ^{125}I -iododeoxyuridine as well as ^3H -methyl thymidine. Ms. Sailstad stated that there was unanimous support of the Panel conclusions among the IWG and ICCVAM. However, the IWG recognized the need to use traditional guinea pig tests for metal compounds and in laboratories where radioisotopes can not be used or are not feasible. IWG tasks included providing a standard protocol to agencies. As identification of the auricular node is difficult, two diagrams of its location are included in the protocol. The Panel report is complete, as is the retrospective audit, and the conclusions of the Panel and the IWG have been forwarded to the Federal agencies. Ms. Sailstad concluded her presentation by acknowledging the support of the IWG members and others involved in the process.

It was asked if there was a reference in the literature to the auricular node. Ms. Sailstad replied that the auricular node was not mentioned in any of the anatomy references she could find, but that the term is used in all of the LLNA articles. Ms. Sailstad mentioned that a pathologist conducting a necropsy with her on treated mice had called it the parotid lymph node; however, other consulted pathologists called it the superficial cervical node.

Several individuals, including Dr. Rebecca Dearman (Zeneca) and Ms. Cindy Ryan (Procter and Gamble), confirmed to Ms. Sailstad that their LLNA studies were conducted using the same node. Ms. Sailstad concluded that, despite what it is called at this point in time, the scientific community is dissecting out the same node. Since it would be difficult for an untrained person to locate the correct lymph node in unsensitized animals, it was recommended that they should practice with colored agents and/or a strong sensitizer.

Dr. Faustman asked about the weight given to guinea pig test data and asked if the stimulation index (SI) threshold would have been different had the human data been given more weight. NTP Center staff responded that the Panel gave more weight to the guinea pig database because of the greater number of chemicals tested and because the human data base contained a disproportionate number of positive responses. Another question dealt with the adequacy of the interlaboratory assessment, as one laboratory appeared to have data quite different results compared with the others. The response was that an adequate assessment had been conducted, and that the apparent difference may have been due to the activity of the radioisotope used.

Dr. Hurt asked how smoothly and rapidly the LLNA would be accepted by Federal agencies. Ms. Sailstad answered that Dr. Wilcox, the next speaker, would discuss that topic, but that there was great hope that the agencies would respond quickly, because of the tremendous effort put in by many agency staff members. Dr. Hurt stated that the success of ICCVAM will be determined, at least in part, by what happens with the LLNA.

Food and Drug Administration (FDA) Consideration of ICCVAM Test Method Recommendations

Dr. Wilcox gave a presentation on FDA regulatory acceptance and implementation of ICCVAM recommended test methods. The FDA is developing a formal process for implementation of and/or response to methods forwarded by ICCVAM. Dr. Wilcox outlined the process from the point when an Agency receives a method to FDA agency-wide implementation. He explained the difference between acceptance (no formal definition) and implementation. Implementation would depend on many factors, such as relevance to the activities of the particular FDA Center. He emphasized the importance of Agency representation on the working groups. When such representation occurs, then Agency representatives have an opportunity to provide input throughout the ICCVAM process, so that acceptance and implementation should progress more smoothly.

Dr. Wilcox indicated how the multiple FDA Centers were involved in the process, and discussed how the ICCVAM committee recommendations are disseminated throughout the various FDA Centers and Office of Regulatory Analysis (ORA). Each Center has a representative on the FDA Toxicology Panel. The Office of Science (OS) prepares a response summarizing the Centers' implementation plans. The FDA response is

authorized by the Office of the Commissioner (OC) and the FDA response is conveyed to NTP/NIEHS.

Corrositex® Test Method Peer Review

Overview of the Review Process

The Corrositex peer review meeting was held on January 21, 1999. Dr. Stokes presented an overview of the review process, including the history of the interaction between ICCVAM and InVitro International, Inc. (IVI), the producers of Corrositex, which dated back to 1995. The members of the Corrositex Working Group (CWG) represented 13 Federal agencies. The Peer Review Panel (Panel) consisted of 11 scientists, and was chaired by Dr. Robert Scala. The two principal charges given to the Panel were: (1) to evaluate the extent to which the submission addressed the ICCVAM validation and regulatory acceptance criteria; and (2) to develop consensus/majority conclusions on the usefulness of Corrositex for assessing dermal corrosivity potential. The components of the evaluation guidance developed by the CWG were presented. Dr. Stokes reviewed the agenda of the public meeting and discussed activities needed to complete the review process. He estimated that the draft Panel report would be distributed to the Panel for review in the next few weeks. When finalized, the report will go to the CWG for comment and recommendations. The CWG will then forward the report to ICCVAM with a request that they forward the document to the Federal agencies.

Dr. Stokes briefly reviewed the existing definitions of corrosion, including the OECD harmonized definition. Dr. Stokes explained the OECD tiered testing approach to the evaluation of dermal corrosion and irritation potential. Dr. Stitzel asked whether both positive and negative responses in Corrositex required further testing. Dr. Stokes stated that negative responses required further testing for dermal irritation except for certain chemical classes for transportation purposes.

Overview of the Method

Dr. Goldsworthy gave a brief presentation on the Corrositex assay protocol. This *in vitro* assay measures the potential corrosivity of test substances by measuring penetration and/or diffusion through a biobarrier system. Substances that do not cause a pH change in the chemical detection system (CDS) do not qualify for the assay. Dr. Goldsworthy provided a Corrositex test kit for review, outlined each test step, and listed the laboratory equipment necessary to perform the test. The test is relatively inexpensive to conduct; the suggested retail cost of the assay is \$575 for a two-sample kit and \$995 for a four-sample kit. A trained technician can easily test eight samples per day. Dr. Faustman asked what the difference was between category 1 and category 2

chemicals and why these categories were assigned. Dr. Goldsworthy explained that the categorization step is used to determine which chart to use in assigning UN packing groups. The two categories reflect whether the chemical induced a change to either acidic or basic conditions, respectively, in the chemical detection system. He mentioned that the categorization step does not reflect any mechanistic basis, as the test is empirically designed.

Peer Review Panel Conclusions and Perspectives

Dr. Green reviewed the deliberations of the Panel that evaluated the sufficiency of the test method description (including the protocol), concentrating on the scientific basis. The general conclusion was that the test method description was sufficient, but there were several concerns. These included (1) the composition of the biobarrier; (2) the correlative, not mechanistic, nature of the test; and (3) the range of test applications (types of materials suitable for testing). The group also evaluated whether there was agreement between the protocol used in the validation studies and the current protocol. It was concluded that the protocol used to generate the submission data was in general agreement with the current protocol, with several minor differences noted. These included differences in the maximum shelf-life of the prepared biobarrier (reduced from ten to seven days); the time for classification of category 2 materials for packing group III (increased from 30-45 to 30-60 minutes); and for Category 2 noncorrosives, determining the time limit that a material had to be placed in the biobarrier system (increased from 45 to 60 minutes) to be classified as noncorrosive.

Dr. Green then compared the Corrositex protocol used by IVI in generating the Submission data and the one used by ECVAM during the ECVAM-sponsored validation studies. Two differences were highlighted. First, the IVI refrigeration time prior to using the biobarrier was two hours, whereas the ECVAM refrigeration time was overnight. Second, IVI used nitric or sulfuric acid as a positive control whereas ECVAM used sodium hydroxide. These differences were not considered critical to the evaluation process. He then presented concerns raised by Panel members regarding dose and breakthrough time selection. Most Panel members thought that these were appropriate; however, one member thought that there was little to no rationale for the doses used other than mimicking those used in the animal study, and that the breakthrough times were set to achieve results consistent with regulations. The Panel generally concluded that the test method protocol was adequate and complete. The Panel felt that the strengths of the assay were that it is simple and rapid and that it is less expensive than the corresponding animal assay. Its limitations were the high number of non-qualifying substances, the high number of false positives and negatives in certain chemical categories, and that it could not be used in many circumstances as a replacement for the animal assay due to those two limitations. The Panel concluded also that the assay could not be used to predict the corrosivity of complex mixtures since composition information on these mixtures was considered confidential and was not provided.

A member of the public asked Dr. Green what the Panel thought about the tight time range for assignment of packing groups for Category 1 materials. Dr. Green stated that the Panel did not address this issue.

Dr. Hurt asked for clarification on how this assay would be used as a screen. Dr. Green responded that it depends on the chemicals involved. In general, chemicals that test positive in Corrositex require no further testing and may be labeled as corrosive, while negative responses must be evaluated *in vivo* for dermal irritation. In some cases, however, an investigator may wish to proceed with additional testing for a positive test result. Dr. Theran commented that Corrositex was intended to be a scientifically-based test and that one of its strength is that it does not use animals.

Dr. Hayes continued the presentation by discussing test method description, performance, data quality, reliability, other considerations, and related issues. He noted that the Panel struggled with whether the sponsor gave sufficient information for an adequate evaluation. Dr. Hayes cited inadequacies in data presentation and reviewed the performance characteristics of the assay. The Panel concluded that the performance characteristics, particularly for acids and bases, were good. The IVI studies were not conducted in accordance with strict Good Laboratory Practice (GLP) guidelines; however, NTP Center staff and NTP Quality Assurance audits concluded that the outcome of the testing results was not affected by any of the issues identified in the Quality Assurance audit. Dr. Hayes briefly mentioned other corrosivity test methods, including the rat skin TER assay, EpiDerm, and Episkin.

Dr. Green concluded with a brief summary of the Panel conclusions. The Panel felt that the method could be used as part of a tiered approach to identify some dermal corrosives. Corrositex could be used as a stand-alone assay for organic and inorganic acids and bases in specific testing situations (e.g., U.S. Department of Transportation). As far as adequacy for determining the absence of dermal corrosion, the Panel felt that acids, acid derivatives, and bases may be correctly identified, and that Corrositex may be equivalent to the rabbit test for these chemical classes. All other chemical classes should be tested in a tiered approach. Finally, with regard to the reduction, refinement, and replacement of *in vivo* tests, Corrositex does not meet the criteria for complete replacement due to the limited number of chemical classes for which it is applicable. However, Corrositex does, under certain instances, allow for reduction and refinement of animals.

Dr. Theran asked for comment on the limited number of chemicals that qualify for the test. Dr. Green stated that, simplistically, the chemicals that do not induce a change in pH to <4.5 or >8.9 do not qualify.

Dr. Theran asked why the chemical database was described as limited and how the data was evaluated. Dr. Hayes replied that the many of the individual chemical classes consisted of too few chemicals for the Panel to feel comfortable with the use of this assay for those classes. Dr. Stitzel asked if that was due in part to the fact that the test is not mechanistic. Dr. Green answered that this was indeed the case. Dr. Montgomery asked if any of the other *in vitro* assays were mechanistically based. Dr. Hayes replied that Episkin appeared to be.

Dr. Stitzel asked if there was any consideration given to the fact that this was a patented system. Dr. Hayes said that this issue was not discussed but that patented methods are not acceptable as a test method under OECD guidelines. Dr. Stitzel commented that some methods accepted by OECD have patented components.

An ACATM member commented on the “spirit of GLP” and the credibility of the data. He asked whether there should be guidelines for conducting studies in the spirit of GLP. Dr. Goldsworthy commented that the data were not generated under GLP, but were considered to be credible and that other data sets were also looked at by the Panel. The ICCVAM validation guidelines do not state that test data must be collected using GLP, but that information on how the studies were conducted aids the Panel in their deliberations.

Dr. Hurt asked if it was possible for the test to be validated for complex mixtures in the future. Drs. Green and Hayes both replied that it could be. Dr. Goldsworthy added that the concordance for mixtures was good, but that it is not feasible to evaluate the predictive capacity of the test for mixtures when their composition is not known.

Potential Partnership Opportunities for ICCVAM/NTP Center

EPA Endocrine Disruptor Testing and Screening Methods

Dr. Tony Maciorowski gave a speaker-phone presentation on the EPA Endocrine Disruptor Screening and Testing Initiative. In August, 1998, EDSTAC provided a report that included recommendations on the use of high throughput automated robotic prescreens to set priorities; a relational database to set priorities and track data; eight screening assays to detect potential estrogenic and androgenic effects; and five tests using mammals, birds, fish, frogs, and shrimp to verify and characterize adverse effects. EDSTAC also recommended validation of all screens and tests prior to regulatory implementation.

The proposed screening battery includes three *in vitro* tests (i.e., estrogen and androgen receptor binding or reporter gene assays, and the steroidogenesis assay with minced testis) and five *in vivo* tests (i.e., rodent three-day uterotrophic assay; rodent 20-day pubertal female with thyroid assay; rodent five- to seven-day Hershberger assay, frog

metamorphosis assay, and the fish gonadal recrudescence assay). The two receptor binding or reporter gene assays are proposed as high throughput prescreening (HTPS) methods. Alternative screens recommended by EDSTAC include the *in vitro* placental aromatase test and the *in vivo* modified three-day uterotrophic test, the 14-day intact adult male with thyroid assay, and the 20-day pubertal male with thyroid assay. The proposed testing battery includes multigenerational and developmental studies using rodents, birds, frogs, fish, and shrimp.

Dr. Maciorowski reviewed the EPA implementation activities for the screening and testing validation program. Within a two- to five-year phased time frame, EPA plans to implement the standardization and validation of the proposed screening and testing assays. During this time, EPA plans to develop prevalidation and validation data for ICCVAM peer review. Four workgroups focusing on *in vitro*, *in vivo*, ecotoxicity, and chemical management have been established to consider the validation scheme; a statistical support team is also in place to assist these workgroups.

HTPS has been under scrutiny and discussion. Sixty-one chemicals were selected to demonstrate weak and strong agonist and antagonist activity. Because of a large number of assays and a limited budget, EPA's validation strategy is to focus on prevalidation of pubertal female, pubertal male assays in FY99; develop *in utero* through lactation screen (FY2000); rely on OECD efforts for validation of uterotrophic and Hershberger assays; and to validate non-mammalian screens and Tier 2 tests in FY2001. The pubertal male and female protocols are ready for review by the Task Force *In Vitro* Working group in March, 1999. Dr. Maciorowski summarized the current and likely future work of the OECD.

Dr. McClellan asked what consideration has been given to identifying specific chemicals for which solid data are available in terms of information on endocrine activity. He added that this is essential in order to proceed with validation efforts. Dr. Maciorowski responded that it has been done partially (i.e., literature searches, on-going workshops such as one being supported by the Chemical Manufacturing Association). They have also investigated what assays are available and the chemicals that have been used in those assays. The uterotrophic and Hershberger assays have been used for many years and extensive data are available. Dr. Maciorowski stated that these assays were first used for screening pharmaceuticals, and that there is less information on chemicals inducing a weak response. He stated that there is a list of 10 to 12 chemicals with known activity in these assays.

Dr. McClellan also asked about the standardization and validation task force workgroups, and to what extent EPA has given priority to public communication workgroups to ensure that it is an open process. Dr. Maciorowski stated that this has been a topic of discussion during the EDSTAC process. EPA is in the process of developing public workshops, public comment notices, etc. and is also developing an endocrine disruptor

database as a repository for information. He added that the EPA is following the EDSTAC recommendations to keep the information publicly available.

Dr. Denison asked how a high throughput assay is defined. Dr. Maciorowski answered the intent was to expand the use of assays that are being conducted for drug development. Dr. Denison also asked if estrogen and progesterone receptor hormone systems were being considered also. Dr. Maciorowski answered that these assays would require further development.

Dr. Stitzel asked if the workgroups have at least one member that really understands the validation process in order to avoid unnecessary and wasted effort. In response, it was indicated that close liaison is anticipated between the workgroups and Drs. Stokes and Hill on how the workgroups should interact to ensure that ICCVAM principles are followed. However, currently, there is no direct liaison with ICCVAM. Dr. Stokes commented that ICCVAM involvement will help the process by informing work groups of the information that must be provided for the peer reviews.

Dr. Lucier asked if the OECD would be validating the uterotrophic and Hershberger assays; Dr. Maciorowski replied that it would and that there is no anticipated role for ICCVAM for conducting the needed studies. Dr. Maciorowski also stated that they were attempting to reduce redundancy. Dr. Lucier asked again about the uterotrophic and Hershberger assays and whether it would make sense for ensure that the OECD efforts are consistent with the ICCVAM submission guidelines. Dr. Maciorowski stated that there were competent scientists representing the U.S. as part of the OECD and that these scientists are interested in the same scientific principles.

Dr. Hurt asked which of the assays in the Tier 1 and Tier 2 screens does the validation task group envision bringing through the ICCVAM process. Dr. Maciorowski responded by stating that all of them would be brought to ICCVAM to some extent.

Dr. Green asked about the OECD validation management committee and was told that it is a subgroup of the OECD Endocrine Disruptor Testing Activity (EDTA).

Dr. Theran asked if 87,000 was the number of chemicals are being evaluated. The reply was that this is the number that will be considered in the initial sorting base, but that approximately 25,000 of the compounds were polymers that will probably not be tested. Approximately 2500 more are inert and also won't be tested. Basically, only a subset of the 87,000 chemicals discussed will be investigated. Dr. Theran also inquired on efforts to reduce the number of animals being used for testing. Dr. Maciorowski replied that existing information (toxicity test results, product chemistry, etc.) will be used to reduce the number of chemicals tested thereby reducing animal use. At this point, he mentioned that about 15,000 chemicals may be tested, although it is not known how many of those will be investigated using animals.

Susan Hurt asked how the standardization/validation task force would view ICCVAM forming an interagency working group to foster the validation of the *in vitro* methods. Dr. Maciorowski stated that there will be representatives from multiple agencies on the task force. Dr. Hurt stated that, looking at it from the perspective of the ICCVAM structure, these working groups would act more like sponsors. Dr. Maciorowski stated that the EPA views it differently, and considers the Agency to be the sponsor and the workgroups to be the reviewers.

Dr. Hurt mentioned to ACTAM that Penny Fenner-Crisp, EPA, was part of the endocrine disruptors group and had expressed interest in working with ICCVAM in the past. She mentioned that Dr. Fenner-Crisp should be contacted and asked for her position on ICCVAM involvement.

Action: ACATM unanimously agreed that Drs. Lucier and Stokes should contact Dr. Fenner-Crisp and ask that the EPA task groups work with an ICCVAM workgroup to coordinate assay validation.

EPA High Production Volume (HPV) Chemical Testing

Mr. Charles Auer gave a speaker-phone presentation on the Vice President's Chemical Right-to-Know Initiative, and the HPV Challenge Program initiated by the EPA Office of Pollution Prevention and Toxics. Three new initiatives aimed to strengthen the public's right and ability to know about the possible health and environmental risks from chemicals are being undertaken. These included:

- Increased screening level testing for about 2800 HPV chemicals through voluntary efforts and EPA-drafted rules;
- Pursuing rules to ensure that chemicals to which children are exposed are fully tested for their health effects; and
- Proposing lower reporting thresholds for persistent and bioaccumulative chemicals on EPA's Toxic Release Inventory (TRI).

A preliminary screen by EPA of 2,863 industrial chemicals with production or import volumes greater than 1,000,000 lbs. per year in the United States found that 43% of the U.S. HPV chemicals have no publicly available studies for any of the six basic endpoints (acute toxicity, chronic toxicity, developmental and reproductive toxicity, genotoxicity and mutagenicity, ecotoxicity, and environmental fate). Further, only 7% of the U.S. HPV chemicals have a full set of publicly available studies for these endpoints. The HPV chemical challenge is currently a volunteer program for manufacturers and importers to test their HPV chemicals before 2004. A rule will be written by 1999 for the chemicals that no one volunteers to test. Mr. Auer then explained the steps that a "volunteer" would need to conduct to complete testing for the six basic endpoints. The manufacturer

or importer would first volunteer to test a specific chemical, and would then collect, assess, and write robust summaries of all available data on the chemical. Using this information, the manufacturer or importer would develop a testing plan, and submit it to EPA with the summary data. Testing would then be conducted for the basic endpoints for which there are data gaps, according to the OECD Screening Information Data Set (SIDS). SIDS provides a defined battery of tests that are considered to be effective for collecting data on six basic endpoints. The manufacturer or importer would then revise the summary to include the new testing data.

Efforts to refine, replace, and reduce animal use have been considered as part of this initiative. To decrease the need for animal testing, participating companies search literature and company files for relevant existing data that could be used before they initiate a testing strategy. The EPA also encourages the development of information on chemical categories that can be used, as well as the use of structure activity relationships (SAR) to estimate potential chemical effects. With respect to refinement of animal testing, EPA supports measures to ensure humane care and upkeep of laboratory animals, and supports the use of principles on humane endpoints as described in an OECD guidance document under development. The EPA supports the replacement of animal testing by allowing the use of a bacterial assay for gene mutation testing, and by accepting *in vitro* test results for detection of chromosomal aberrations; however, *in vivo* methods are preferred for new testing of this endpoint. To reduce animal use, the EPA allows the use of the up-and-down acute toxicity test (OECD guideline 425) in place of the traditional LD₅₀ study (OECD guideline 401), which uses more animals. EPA also recommends that repeat dose, reproductive and developmental toxicity testing be combined to reduce the number of animals tested. EPA is also investigating the use of a portion of the animals used for testing under OECD guideline 422 for use in an *in vivo* micronucleus study (OECD guideline 474). Mr. Auer stated that these efforts could reduce the animals tested per chemical by 70% compared to the use of OECD guidelines 401, 407, 414, 415, and 474.

Further information on this EPA initiative, and on the chemicals for which information is known, is available on the internet (www.epa.gov/chemrtk) or by calling the TSCA hotline (202-554-1404).

Dr. Bailey asked for an additional explanation on what is meant by “adequacy of existing data.” Dr. Hernandez, on the speaker phone with Mr. Auer, replied that the phrase refers to experimental adequacy as well as pertinence of the data to the endpoint. Mr. Auer added that EPA does not require that data are collected under GLP, as long as the data is deemed to be reliable, and that the goal is to provide adequate screening-level information on the chemicals.

Dr. Hurt asked how the proposals for the reduction of animal testing were accepted at the recent OECD meeting. Mr. Auer stated that, in general, the OECD response was

favorable. The use of the up-and-down method for acute toxicity testing was recommended. However, the European Community prefers that the 28-day, one generation, and developmental toxicity studies be conducted separately. Their rationale is based on the difficulty in setting doses appropriately for multiple testing schemes.

Dr. Bailey mentioned that the 28-day study protocol recommended by OECD calls for administration of a 1000 mg/kg dose, and asked if this is acceptable to the EPA. Mr. Auer answered that testing at this dose level is acceptable if the results of such testing do not indicate that further testing is needed.

Dr. Stitzel stated that there is interest in evaluating *in vitro* data by comparison to acute oral toxicity data, and asked for the EPA position in this regard. Mr. Auer stated that EPA would accept such *in vitro* data if it could be demonstrated to be as reliable as the *in vivo* data, but that he was skeptical that this level of performance could be achieved with an *in vitro* test.

Dr. McClellan asked for the EPA position with regard to collecting tissue samples of animals tested under the HPV chemical challenge initiative. He felt that such samples could either be stored for future testing or tested promptly, and results could be compared to *in vivo* testing results to allow for *in vitro* assay optimization. If a data bank of information on these methods was collected and evaluated in this manner, then the assay may have potential as a future replacement of the *in vivo* test, although reporting results at this early stage may be detrimental to the company at this point.

Dr. McClellan asked if the EPA would require the reporting of data collected by this testing method. Mr. Auer stated that he could not answer that question. Dr. McClellan further explained that, in conducting testing for the HPV challenge, animals are being dosed repeatedly, and that animal rights activists would like to eliminate further testing of this sort. He felt that if these analyses could be conducted and reported based on a microchip or other *in vitro* methods, that it would be a positive step with respect to animal welfare, and may open the door to the development of new approaches. Mr. Auer asked that Dr. McClellan write to the EPA recommending that the agency examine and consider this issue, and that the agency would give it their attention. Dr. Stitzel mentioned that the committee would draft and send the letter.

Dr. Theran asked why the EPA insists on using *in vivo* genetic toxicity tests when OECD has been allowing the use of *in vitro* technology. Mr. Auer answered that OECD began accepting both *in vivo* and *in vitro* genetic toxicity testing in the early 1990s, but that based on an analysis of the genetic toxicity database, EPA believes that the *in vivo* data is more useful.

Future Directions

Topics of discussion for future directions include:

- “Spirit” of GLP and setting a foundation for validation;
- Reciprocity of validation with ECVAM; and
- Acceptance of data

Public Comment

Mary Beth Sweetland of PETA asked Dr. Wilcox to state the potential lag time from the time the FDA begins discussions about a test method to the time the commissioner approves the letter. Dr. Wilcox answered that the anticipated response time is July for the LLNA, but expects most responses before that time. Ms. Sweetland asked if it is possible for an individual reviewer to recommend that a test method not be used when approving an application. Dr. Wilcox responded that a reviewer will have that option. Ms. Sweetland commented on Corrositex , saying that she would like to see that test method accepted, because of the high concordance and applicability to specific chemicals. Lastly, she commented on endocrine disruptors and that the EPA validation strategy is ill-conceived because there are problems with using animals to predict endocrine disruption.

A member of the public asked if the USDA tracks the number of guinea pigs used. Dr. Stokes answered yes, but there is no requirement to track the number of rats and mice because mice and rats are not covered by current USDA Animal Welfare regulations..

Adjournment

The meeting adjourned following a brief discussion of the next meeting date.